IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-729 MIS:as

In re patent application

No.	08/931,721
Applicant:	Barbara Papadopoulou et al
Title:	MACROPHAGE-INFECTING PARASITES EXPRESSING A GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR
Filed:	September 16, 1997
Group No.	1644
Examiner:	R. Haynes

April 25, 2000

APPEAL BRIEF

BY COURIER

The Commissioner of Patents
and Trademarks,
BOX AF,
Washington, D.C. 20231,
U.S.A.

Dear Sir:

1. Introduction

This Appeal Brief is submitted in support of the appeal from the Final Rejection of Claims 1, 3 to 7, 9, 10 and 21. The form of the appealed claims is found in the Appendix hereto. Three copies of this Appeal Brief are submitted.

2. Extension of Time

Petition is hereby made under the provisions of 37 CFR 1.136(a) for an extension of one month of the period for filing an Appeal Brief on this case. We enclose our cheque in the amount of the prescribed fees.

3. Real Party in Interest

The real party in interest with respect to this patent application is Université Laval to whom the inventors have assigned the invention. An Assignment has been submitted to the PTO for recordal.

4. Related Appeals and Interferences

The appellant, appellant's legal representatives and assignee are unaware of any other appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

5. Status of Claims

This application was filed with twenty claims. In response to the first Office Action, claims 2, 8 and 11 to 20 were deleted, claims 1, 3, 5, 6 and 7 were amended and new claim 21 added. The form of the claims appealed appears in the Appendix.

6. Status of Amendments

Submitted herewith is an Amendment after Final Action, rewriting claim 10 in independent form. Entry of such Amendment should have the effect of allowance of such claim and withdrawal of such claim from this Appeal.

7. Summary of Invention

The invention is concerned with a certain novel macrophage-infecting parasite which is a strain of *Leishmania* which expresses a granulocyte macrophage colony stimulating factor (GM-CSF) gene, as well as attenuated forms of such parasite in which at least one gene contributing to virulence is functionally disabled (page 4, lines 11 to 36).

8. Issues

The issues for consideration in this Appeal are:

(a) Claims 1, 3 to 6 and 21 are rejected under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al.

(b) Claims 1, 3 to 7 and 21 are rejected under 35 USC 103(a) as being unpatentable over Moore et al, in view of Wong et al, and further in view of Laban et al.

(c) Claim 9 is rejected under 35 USC 103(a) as being unpatentable over Moore et al in view of Matlashewski et al.

9. Grouping of Claims

It is submitted that, for the reasons advanced below, that the claims do not stand or fall together.

10. Argument

(a) Nature of the Invention

As noted above, the invention relates to a macrophage-infecting parasite which is a strain of *Leishmania*, which may be selected from the group consisting of *Leishmania donovani*, *Leishmania braziliensis, Leishmania tarentolae*, *Leishmania major*, *Leishmania mexicana*, *Leishmania tropica* and *Leishmania aethiopica*, preferably *L. donovani* and *L. major* (claims 1 and 21). The parasite has been genetically modified by transformation with a plasmid containing the GM-CSF gene, so that the parasite expresses GM-CSF (claim 21). The GM-CSF may be of murine (claim 5) or human origin (claim 6). The GM-CSF gene preferably is expressed from the modified parasite using the α-tubulin intergenic sequences of *Leishmania enrietti* (claim 7).

The parasite preferably is provided in an attenuated form with a reduced ability to infect or survive within macrophages (claim 4). In particular, at least one gene of the parasite contributing to virulence thereof is functionally disabled (claim 9).

(b) Rejection of Claims 1, 3 to 6 and 21 under 35 USC 103(a)

The Moore et al reference is concerned with a study of apoptosis of bone-marrow macrophages (or BMMs). As set forth in the introduction to the paper, in the absence of macrophage CSF, BMMs undergo a rapid decline in cell viability, resulting in the death of approximately 70% of the population within 48 hours and that BMMs infected with *L. donovani* demonstrate enhanced viability in the absence of growth factor. As the authors note, these findings imply a soluble factor was secreted by *L. donovani* infected macrophages to the culture medium that may act in an autocrine manner to prevent cell death by apoptosis (see p. 2932, right-hand column, last para.)

The paper goes on to describe RT-PCR characterization of the cytokine expression profile of BMMs infected with *L. donovani*. This study found that the infection of BMMs by *L. donovani* promastigotes induced expression of four cytokine genes, namely GM-CSF, TNF-α, TGF-β and IL-6 (p. 2933, left-hand column). *L. donovani* infection of BMMs did not stimulate the expression of the M-CSF gene. Following the finding of expression of these cytokines by the BMMs, the authors studied whether any of the cytokine gene products could inhibit apoptosis of

the BMMs and, to this end, BMMs (uninfected) were treated with recombinant versions of the cytokines. The authors found that rTNF-α and rGM-CSF were capable of inhibiting apoptosis, as demonstrated by a reduced level of fragmented DNA in the treated BMMs.

The next step in the study reported by Moore et al was to determine whether *L. donovani*-infected BMMs secreted TNF-α and GM-CSF into the culture medium. It was found that a significant level of TNF-α was present in the infected cell culture supernatants but no GM-CSF was detected (see p. 2933, right-hand column, last para.). Thus, while *L. donovani*-infected BMMs express the GM-CSF gene (as determined by the RT-PCR tests), the authors were unable to detect GM-CSF protein in supernatants from infected cells.

From these results, it was concluded by the authors that it is the secretion of TNF-α from the infected cells which is responsible, along with other factors, for inhibition of apoptosis of *L. donovani*-infected BMMs in the absence of M-CSF. The authors conclude their study with the following:

> "... two major observations are reported in the present study that we believe are related. The first is that *L. donovani* infection prevented apoptosis in BMMs and that this could also be mediated in part by LPG. Second, infected BMMs express a number of cytokine genes, which probably contributes to the prevention of apoptosis and may also be important in other aspects of the infection process. The enhancement of host cell viability could facilitate the spread of infection by increasing the number of host cells available for parasitization by *L. donovani* and by increasing the number of circulating infected macrophages for uptake by the sandfly vector."

Thus, the whole study presented by Moore et al relates to infection of BMMs by *L. donovani* and the effect of such infection on cell apoptosis of the BMMs. There is no hint or suggestion of any modification to the *L. donovani* itself to permit the *Leishmania* to express a foreign gene. The present invention, by contrast, provides a macrophage-infecting parasite, which is a strain of *Leishmania* and which is transformed by a plasmid containing a granulocyte macrophage colony stimulating factor (GM-CSF) and which parasite expresses GM-CSF.

Claim 1 requires the provision of a macrophage-infecting parasite which is a strain of *Leishmania*. Thus, the parasite is one which is a strain of *Leishmania* and is macrophage infecting. In Moore et al, the BMMs are infected by a parasite which is a strain of *Leishmania*. The *L. donovani* which infects the BMMs

does not express GM-CSF as well as the other cytokines IL-6, TNF-α and TGF-β mentioned in Moore et al, but rather it is infection of the BMMs by *L. donovani* which induces the BMMs to express such genes.

It is submitted that the Wong et al reference does not remedy these defects of Moore et al as a primary reference. The Wong et al reference teaches the GM-CSF gene and its recombinant expression. Simply because there are no detectable levels of GM-CSF in the supernatants of BMMs infected with *L. donovani* provides no motivation for there to be such expression, since such is merely an observation of an experiment without conclusion as to its effect. BMMs infected by *L. donovani* are induced to express, from the BMMs, four cytokine genes, including GM-CSF, provides no motivation to transform a strain of *Leishmania* with a plasmid containing a GM-CSF gene so that the strain of *Leishmania* itself expresses GM-CSF. There is no motivation whatsoever provided by the Moore et al teaching to achieve detectable levels of GM-CSF protein.

In the Final Action, the Examiner asserts:

> "Additional motivation resides in that such transformation of *Leishmania donovani* with a GM-CSF expression plasmid would more efficiently prevent apoptosis of BMMs which, therefore, increase BMM survivability and viability (pg. 2935, last two paragraphs, and last two paragraphs, pg. 2935); and thereby decrease overall *Leishmania* infectivity and survivability..."

There is no such motivation provided. The cited portions of the paper merely state that cellular cytokine gene expression from the BMMs was induced by infection by *L. donovani* and that such cytokines include GM-CSF. As already noted, the authors of Moore et al conclude that the enhancement of host cell viability could facilitate the spread of infection rather than decrease overall *Leishmania* infectivity and survivability, as suggested by the Examiner.

Accordingly, it is submitted that the only macrophage-infecting parasite described by Moore et al is *L. donovani* and the paper describes the effect of infection of BMMs by this macrophage-infecting parasite on cell apoptosis. While there is an observation that such infection induces expression of cytokines, including GM-CSF, by the BMMs, but that GM-CSF is not detected in supernatants of the parasite-infected BMMs, there is no motivation whatsoever to transform the macrophage-infecting parasite with a plasmid containing the GM-CSF gene so as to permit expression of GM-CSF by the parasite, as claimed in claims 1 and 21.

The Examiner includes claim 4 in this rejection. Claim 4 requires that the parasite have a reduced ability to infect or survive within macrophages. There is no suggestion in Moore et al of any such further modified form of the *Leishmania* parasite and hence this claim should be considered to be separately patentable from the remaining claims contained in this rejection.

Having regard to the above, it is submitted that the Examiner clearly is in error in rejecting claims 1, 3 to 6 and 21 under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al.

(c) Rejection of claims 1, 3 to 7 and 21 under 35 USC 103(a)

In this rejection, the Examiner adds the Laban et al reference to the combination of Moore et al in view of Wong et al. The relevance and deficiencies in the combination of the Moore et al and Wong et al references to the patentability of claims 1, 3 to 6 and 21 have been discussed above and requires no further elaboration.

The Laban et al reference is cited by the Examiner for the teaching of the expression of the neomycin gene using the α-tubulin intergenic sequences of *L. enrietti*. This is the sequence defined in claim 7. The Laban et al reference is silent with respect to the possibility of utilizing such sequences for expression of any other gene sequences beyond the neomycin sequence specifically discussed therein. In any event, it is submitted that the Laban et al reference provides no teaching which would remedy the basic defect of the combination of Moore et al and Wong et al discussed above and provides no motivation to transform a strain of *Leishmania* to achieve expression of GM-CSF from the *Leishmania* strain.

Accordingly, it is submitted that the Examiner clearly is in error in rejecting claims 1, 3 to 7 and 21 under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al and further in view of Laban et al.

(d) Rejection of claim 9 under 35 USC 103(a)

In this rejection, Moore et al is cited in combination with Matlashewski et al. Claim 9 is dependent on claim 1 and recites that at least one gene of the parasite contributing to its virulence has been functionally disabled. This modification is in addition to the modification to the *Leishmania* strain required by claim 1 and discussed in detail above.

The Matlashewski et al reference teaches the generation of attenuated strains of *Leishmania* and, in particular, to the functional disablement of genes contributing to virulence. It is submitted that there is no disclosure of Matlashewski et al which would remedy the basic defects of Moore et al discussed above and provides no motivation to provide a macrophage infecting parasite which is a strain of *Leishmania* and which is transformed by a plasmid containing a GM-CSF gene and expressing GM-CSF, as required by claim 1.

Accordingly, it is submitted that the Examiner clearly is in error in rejecting claim 9 under 35 USC 103(a) as being unpatentable over Moore et al in view of Matlashewski et al.

11. Summary

Having regard to the above discussion, it is submitted that the rejections of:

(a) Claims 1, 3 to 6 and 21 under 35 USC 103(a) as being unpatentable over Moore et al in view of Wong et al;

(b) Claims 1, 3 to 7 and 21 under 35 USC 103(a) as being unpatentable over Wong et al and further in view of Laban et al; and

(c) Claim 9 under 35 USC 103(a) as being unpatentable over Moore et al in view of Matlashewski et al;

should be REVERSED.

Respectfully submitted,

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